Allelic Variation Within the *Emv-15* Locus Defines Genomic Sequences Closely Linked to the *agouti* Locus on Mouse Chromosome 2

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ABSTRACT

Gene(s) at the *agouti* locus act within the microenvironment of the hair follicle to switch pigment synthesis in the melanocyte between eumelanin (black or brown pigment) and phaeomelanin (yellow pigment). Many phenotypic variants of this locus have been described. The mechanism(s) of gene action causing such variation in coat-color phenotype is not known. The close linkage of an endogenous ecotropic murine leukemia provirus, *Emv-15*, to the *lethal yellow* mutation of the *agouti* locus provides a means to molecularly access genes at or near the *agouti* locus. We have identified and used a unique mouse sequence flanking the *Emv-15* provirus to define three alleles of the *Emv-15* locus. We found a correlation between the presence of specific *Emv-15* alleles and the origins of specific *agouti* locus mutations, confirming close linkage. However, we found some exceptions which suggest that the *Emv-15* locus is closely linked to, but genetically separable from, the *agouti* locus.

THE coat color of mice is affected by several loci, one of which is the *agouti* locus located on chromosome 2 [reviewed by SILVERS (1979)]. Gene(s) at the *agouti* locus regulate a switch mechanism which determines whether eumelanosomes or phaeomelanosomes are produced at any given time by hair bulb melanocytes. Eumelanosomes on which black or brown pigment is synthesized differ ultrastructurally from phaeomelanosomes on which yellow pigment is produced. The molecular basis for the switch mechanism is not understood. The *agouti* locus has been highly conserved throughout evolution and is found in most orders of *Mammalia* [reviewed by SEARLE (1968)].

More than 18 alleles or pseudoalleles of the *agouti* locus have been described in the mouse [reviewed by SILVERS (1979) and GREEN (1981)]. The variants have arisen by spontaneous mutation (A^y, A^{vy}, A^{iy}, A^{sy}, $A^{w\text{-}J}$, A^i, a^{td}, $a^{t\text{-}42J}$, a), chemically induced mutation (a^{16H}), or radiation-induced mutation (A^s, a^m, a^u, a^{da}, a^x, a^{jl}, a^l, a^e). The mechanism(s) of gene action effecting this variation in coat-color phenotype is not known.

The *lethal yellow* (A^y) mutation is dominant over all other mutations of the *agouti* locus. Mice heterozygous for A^y have yellow coats and exhibit an altered susceptibility to spontaneous and induced pulmonary tumors, induced skin tumors, spontaneous reticular neoplasms, spontaneous hepatomas (in males) and spontaneous mammary tumors (in females) [reviewed by SILVERS (1979)]. In addition, heterozygous A^y mice become obese and reproductively impaired with age (GRANHOLM, JEPPESEN and JAPS 1986). Mice carrying any one of the mutations of the *agouti* locus that cause yellow coat color (A^y, A^{vy}, A^{iy}, A^{sy}) become obese and develop mild diabetes-like symptoms [reviewed by COLEMAN (1982)]. Obesity results from an increase in adipose tissue mass caused by fat-cell hypertrophy (JOHNSON and HIRSCH 1972); the rate of lipogenesis fails to decrease post puberty (YEN *et al.* 1976).

Five alleles, A^y (CUENOT 1905), a^x (RUSSELL, MCDANIEL and WOODIEL 1963; PAPAIOANNOU and MARDON 1983), a^l and a^{16H} (LYON, FISHER and GLENISTER 1985) and a^{jl} (L. B. RUSSELL and J. W. BANGHAM, unpublished observations) are homozygous lethal mutations. Homozygous A^y embryos die at day 6 of development before implantation is complete (ROBERTSON 1942). The lethality appears to be a result of a trophectoderm defect (PAPAIOANNOU and GARDNER 1979), possibly affecting trophoblast giant cell differentiation (EATON and GREEN 1963), although arrested blastomeres have been observed (PEDERSEN 1974; CALARCO and PEDERSEN 1976). Embryos homozygous for a^x die at about the same time as A^y homozygotes (PAPAIOANNOU and MARDON 1983). It is not known when in development the lethal action of a^l, a^{16H} and a^{jl} occurs.

Molecular probes for the *agouti* locus can be used to investigate the structure of the gene(s) as well as its mode of action. DNA from inbred mouse strains, congenic or coisogenic for various *agouti* mutations, was screened by Southern blot analysis using a DNA probe specific for ecotropic murine leukemia viruses (MuLVs) (COPELAND, JENKINS and LEE 1983). Results showed that an ecotropic MuLV, *Emv-15*, is closely associated with the A^y mutation carried by strains C57BL/6J-A^y/a, 129/Sv-A^y/A^w, and LT/Sv-A^y/a (COPELAND, JENKINS and LEE 1983).

We report here the cloning of a genomic restriction

fragment containing the *Emv-15* provirus along with 5′ and 3′ flanking mouse sequences from 129/Sv-A^y/A^w genomic DNA. *Eco*RI digestion of the cloned insert gave seven DNA fragments, at least one of which is a unique mouse sequence located 3′ to the *Emv-15* provirus. Genomic Southern blot analysis using this unique sequence probe identified three restriction endonuclease digestion patterns among 15 *agouti* locus genotypes examined. The three restriction patterns identified were, in general, diagnostic of the origin of each *agouti* mutant. However, some exceptions were found which suggest that the *Emv-15* provirus resides at a locus distinct from, but closely linked to, the *agouti* locus.

MATERIALS AND METHODS

Mice: The genotypes of the stocks and strains used are listed in Table 2. The live mice or spleens used were obtained from the animal resource division of The Jackson Laboratory (Bar Harbor, Maine) unless otherwise noted. The exceptions were (1) C3H/Rl, 101/Rl, stock 6PB, and stock 85FBFo were maintained by L. B. RUSSELL; (2) C57BL/6J-A^{vy} mice were a gift from D. W. BAILEY (The Jackson Laboratory); (3) spleens from MOR/Cv mice were a gift from V. M. CHAPMAN [Roswell Park Memorial Institute (Buffalo, New York)]; (4) AEJ/Gn mice were a gift from M. T. DAVISSON (The Jackson Laboratory); (5) strains BALB/cWtEi, C57BL/6J-A^i, C57BL/6J.C3H-A^{iy}, and IS/CamEi were gifts from E. M. EICHER (The Jackson Laboratory); (6) mice carrying the A^{vy} mutation were a gift from A. KANDUTSCH (The Jackson Laboratory); (7) MWT/Le mice were a gift from P. W. LANE (The Jackson Laboratory); (8) spleens from mice heterozygous for the A^y mutation from the YBR/Wi strain were a gift from J. T. NIELSEN [University of Aarhus (Aarhus, Denmark)]; (9) spleens from AG/CamPa and AX/Pa mice were a gift from V. E. PAPAIOANNOU [Tufts University School of Medicine (Boston)]; (10) spleens from YS/Icr mice were a gift from R. J. RIBLET [Institute for Cancer Research (Philadelphia)]; (11) LT/Sv and 129/Sv mice were a gift from L. C. STEVENS (The Jackson Laboratory); (12) spleens from AE/Wf and AM/Wf mice were a gift from G. L. WOLFF [National Center for Toxicological Research (Jefferson, Arkansas)]; and (13) spleens from YBR/Ki mice were obtained from the Kirschbaum Memorial Laboratory Mouse Colony (Rootstown, Ohio).

Genomic cloning and restriction mapping: We identified a 16.7-kb *Bcl*I restriction fragment in 129/Sv-A^y/A^w mice that contains the *Emv-15* provirus by Southern blot analysis (COPELAND, JENKINS and LEE 1983) using an ecotropic virus-specific probe (CHATTOPADHYAY *et al.* 1980). Size-selected 129/Sv-A^y/A^w genomic spleen DNA was cloned into lambda phage EMBL4 (FRISCHAUF *et al.* 1983), and the library was screened with the ecotropic virus-specific probe (MANIATIS, FRITSCH and SAMBROOK 1982). One clone, λ.129-A^y, was identified that contained the *Emv-15* provirus along with 5′ and 3′ flanking mouse sequences. A restriction map of λ.129-A^y was produced as described (MANIATIS, FRITSCH and SAMBROOK 1982; RACKWITZ *et al.* 1984). Five *Eco*RI fragments from the cloned genomic insert of λ.129-A^y were subcloned into the *Eco*RI site of pBR325 (MANIATIS, FRITSCH and SAMBROOK 1982). One subclone, p15.4, contains a unique mouse insert and was used as a probe in Southern blot analyses.

Extraction of DNA: High molecular weight DNA was isolated from mouse spleen or liver tissue as described (JENKINS *et al.* 1982). DNA was extracted from mouse tails by a modification of the protocol of D. HANAHAN (Cold Spring Harbor Laboratory, Cold Spring Harbor, New York). One-half inch of mouse tail was digested overnight at 55° in 600 μl of 50 mM Tris (pH 8.0), 100 mM EDTA, 100 mM NaCl, 1% sodium dodecyl sulfate with 35 μl of 10 mg/ml Proteinase K (Sigma). All subsequent steps were performed at room temperature in 1.5-ml polypropylene tubes. An equal volume of phenol (Fisher) [previously treated with 0.1% 8-hydroxyquinolone and extracted and saturated with 10 mM Tris (pH 8.0)] was added, the mixture was vortexed well, and the phases were separated by centrifugation for 5 min in an Eppendorf Micro Centrifuge, model 5414 (Brinkmann). The aqueous phase (minus interface debris) was transferred to a clean tube and extracted with an equal volume of chloroform:isoamyl alcohol (24:1). DNA was precipitated from the aqueous phase by addition of 2 volumes of 100% EtOH. The DNA was removed with a 5 μl capillary pipette, dissolved in 100 μl of 0.1× SSC (15 mM NaCl, 1.5 mM Na citrate), and stored at −70°. The average yield of DNA from ½ inch of mouse tail was 100 μg.

Southern blot analyses: Restriction endonucleases were obtained from Amersham, Boehringer Mannheim, or New England Biolabs. Restriction endonuclease digestions were performed in low, medium, or high salt buffers (MANIATIS, FRITSCH and SAMBROOK 1982) at temperatures recommended by the suppliers. Genomic DNAs were digested to completion overnight with 5–10 units/μg of the appropriate restriction endonuclease. Conditions for Southern blot analyses were as described (JENKINS *et al.* 1982) except that washes were performed at 65° in 0.2× SSCP, 0.1% sodium dodecyl sulfate. Filters were autoradiographed at −70° with Kodak XAR film and Dupont Lightning Plus intensifying screens. A 1–5-day exposure was usually sufficient for visualization of hybridized fragments.

RESULTS

A brief description of *agouti* locus mutations: Table 1 provides a summation of the relevant features of each *agouti* locus mutation. The gene symbol and common gene name are shown in columns 1 and 2, respectively. The gene name is, in general, synonymous with the coat-color pigmentation pattern. The mode of origin of each mutation is described in column 3. The source and the *agouti* allele that mutated are shown in columns 4 and 5, respectively; they indicate the genetic background on which each mutation occurred. The original alleles that mutated to produce the A^y and *a* mutations are unknown. Both dark-bellied and light-bellied *agouti* mice are commonly observed in feral populations of *Mus musculus* and *Mus domesticus* [reviewed by SAGE (1981)]; for this reason, we have listed both *A* and A^w as wild-type alleles. The a^t mutation was initially found by mouse fanciers. However, since spontaneous mutations from *a* to a^t or A^w are common (SCHLAGER and DICKIE, 1971; SIRACUSA *et al.* 1987), it was not possible to analyze all of the a^t and A^w alleles. Therefore, only the A^w and a^t mutations used in our analysis have been included in Table 1. We have tried to cite the earliest known publication describing each mutation in ref-

TABLE 1

***Agouti* locus alleles**

Gene symbol	Gene name	Mode of origin[a]	Source[b]	*Agouti* allele mutated[c]	Reference	*Emv-15* allele[d]
A^y	*lethal yellow*	S	Mouse Fancy	Unknown	CUENOT (1905)	*v(b)*
A^{vy}	*viable yellow*	S	C3H/HeJ	*A*	DICKIE (1962a)	*a*
A^{iy}	*intermediate yellow*	S	C3H/HeJ	*A*	DICKIE (1966)	*a*
A^{sy}	*sienna yellow*	S	C57BL/6J	*a*	DICKIE (1969)	*b*
A^w	*white-bellied agouti*	W	Feral populations	Wild-type		*a*
$A^{w\text{-}J}$	*white-bellied agouti*	S	C57BL/6J	*a*	Deviant stock, The Jackson Laboratory	*b*
A^i	*intermediate agouti*	S	C57BL/6J	*a*		*b*
A	*agouti*	W	Feral populations	Wild-type	DICKIE (1962b)	*a*
A^s	*agouti-suppressor*	R	(C3H/HeH × 101/H)F_1	*A* or A^w	PHILLIPS (1959, 1966)	ND
a^{td}	*tanoid*	S	C57BL/6J	*a*	LOOSLI (1963)	ND
$a^{t\text{-}42J}$	*black and tan*	S	C57BL/6J	*a*	Deviant stock, The Jackson Laboratory	*b*
a	*nonagouti*	S	Mouse Fancy	Unknown		*b(a,v)*
a^m	*mottled agouti*	R	(C3H/Rl × 101/Rl)F_1	*A* or A^w	RUSSELL (1964)	*a*
a^u	*agouti umbrous*	R	(C3H/HeH × 101/H)F_1	*A* or A^w	PHILLIPS (1976) (M. F. LYON, personal communication)	ND
a^{da}	*nonagouti with dark agouti belly*	R	(C3H/HeH × 101/H)F_1	*A* or A^w	PHILLIPS (1976) (M. F. LYON, personal communication)	ND
a^x	*lethal light-bellied nonagouti*	R	(C3H/Rl × 101/Rl)F_1	*A* or A^w	RUSSELL, MCDANIEL and WOODIEL (1963)	*a*
a^{j1}	*jet lethal*	R	(C3H/Rl × 101/Rl)F_1	*A* or A^w	RUSSELL and MADDUX (1964)	*a*
a^l	*nonagouti lethal*	R	(C3H/HeH × 101/H)F_1	*A* or A^w	PHILLIPS (1976)	ND
a^e	*extreme nonagouti*	R	S strain	*A*	HOLLANDER and GOWEN (1956)	*a*
a^{16H}	*nonagouti-16H*	C	(C3H/HeH × 101/H)F_1	*A* or A^w	LYON, FISHER and GLENISTER (1985)	ND

[a] Abbreviations are S = spontaneous, R = radiation, and C = chemically induced mutations. W indicates the two wild-type alleles *A* and A^w. Spontaneous mutations from *a* to a^t and A^w are common (SCHLAGER and DICKIE 1971; SIRACUSA *et al.* 1987).

[b] Both dark-bellied and light-bellied agouti mice are commonly observed in feral populations of *Mus musculus* and *Mus domesticus* [reviewed by SAGE (1981)]. Both A^y and *a* were originally propagated by mouse fanciers.

[c] The *A* allele is carried by C3H/HeJ, C3H/Rl and C3H/HeH. The A^w allele is carried by 101/Rl and 101/H. The mutated allele could be either *A* or A^w.

[d] ND = not determined. The letters in parentheses indicate the *Emv-15* alleles found as exceptions in only one strain.

erence column 6. The *Emv-15* alleles found in studies described in subsequent sections are listed in column 7.

Identification of a unique mouse DNA sequence flanking the *Emv-15* provirus: The previously determined association of the *Emv-15* provirus with the A^y mutation in strains C57BL/6J-A^y/a, 129/Sv-A^y/A^w, and LT/Sv-A^y/a (COPELAND, JENKINS and LEE 1983) has provided a means to molecularly access sequences at or near the *agouti* locus. Genomic Southern blots using an ecotropic virus-specific probe (CHATTOPADHYAY *et al.* 1980) revealed a 16.7-kb *Bcl*I restriction fragment in 129/Sv-A^y/A^w mice that contains the *Emv-15* provirus as well as 5′ and 3′ flanking mouse DNA (data not shown). Size-selected 129/Sv-A^y/A^w genomic DNA was molecularly cloned into lambda phage EMBL4 (FRISCHAUF *et al.* 1983) and the resultant library was screened with the ecotropic virus-specific probe. One clone, λ.129-A^y, containing the *Emv-15* provirus was identified. A restriction map of the cloned insert is shown in Figure 1A. *Eco*RI digestion of the cloned insert yielded seven DNA fragments, at least one of which (subclone p15.4) is a unique mouse sequence. The p15.4 mouse DNA insert is 1.1 kb long and is located 3′ to the *Emv-15* provirus (Figure 1B).

Three *Emv-15* alleles in inbred mouse strains identified by p15.4: Genomic DNA from inbred mouse strains carrying A^y, A^w, *A*, and *a* was screened by Southern blot analysis with p15.4 to determine whether allelic differences were detectable among these *agouti* variants. A sample of the results obtained is shown in Figure 2. C57BL/6J-*a*/*a* mice exhibit a different restriction endonuclease digestion pattern from C3H/HeJ-*A*/*A* mice when *Kpn*I, *Pst*I, or *Hin*dIII is used. Mice heterozygous for A^y (129/Sv-A^y/A^w and C57BL/6J-A^y/a) exhibit a pattern different from either *A*/*A* or *a*/*a* mice when *Bcl*I, *Kpn*I, or *Eco*RV is used. The restriction patterns of the chromosome carrying A^w in 129/Sv-A^y/A^w mice or 129/Sv-A^w/A^w mice (data not shown) do not vary from those of the chromosome carrying *A* in C3H/HeJ-*A*/*A* mice.

Southern blot analyses revealed three types of restriction patterns characteristic of three *Emv-15* alleles. The three alleles have been designated *Emv-15*[a],

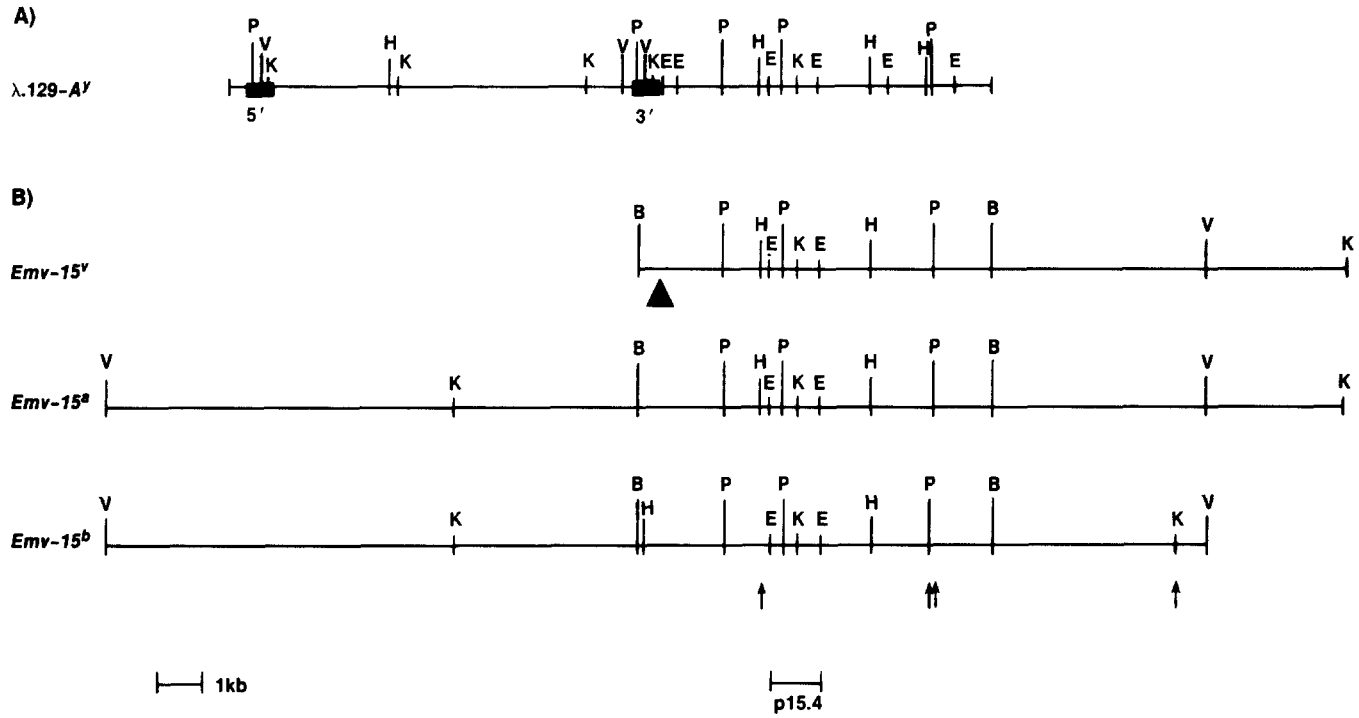

FIGURE 1.—Restriction maps of λ.129-A^y and the three *Emv-15* alleles. The restriction sites for the following endonucleases are shown: B = *Bcl*I, E = *Eco*RI, V = *Eco*RV, H = *Hin*dIII, K = *Kpn*I, and P = *Pst*I. A, The cloned 16.7-kb *Bcl*I genomic insert from λ.129-A^y was mapped as described (MANIATIS, FRITSCH and SAMBROOK 1982; RACKWITZ *et al.* 1984). The genomic insert had lost the *Bcl*I sites on either end during the cloning process. The genomic insert has *Eco*RI sites on either end that are derived from the lambda phage EMBL4 (FRISCHAUF *et al.* 1983) used for cloning. The viral LTRs are shown by *black boxes* and indicate the 5′ and 3′ ends of the *Emv-15* provirus. The p15.4 subclone is shown at the bottom of the figure. B, Restriction maps of the C57BL/6J-A^y and 129/Sv-A^y genomic *Emv-15*^{v} allele, the C3H/HeJ-*A* and 129/Sv-A^w genomic *Emv-15*^{a} allele, and the C56BL/6J-*a* genomic *Emv-15*^{b} allele. Restriction fragment length polymorphisms that defined *Emv-15*^{v}, *Emv-15*^{a} and *Emv-15*^{b} were determined from Southern blot analyses using p15.4 as a probe for digested genomic DNAs from C3H/HeJ-*A*/*A*, 129/Sv-A^y/A^w, C57BL/6J-A^y/*a*, and C57BL/6J-*a*/*a* (Figure 2). The genomic restriction maps are based on a comparison of restriction patterns from the strain DNAs analyzed with each other and with the λ.129-A^y restriction map. Southern blot analyses of these genomic DNAs digested with *Bcl*I and *Kpn*I, *Hin*dIII and *Kpn*I, and *Pst*I and *Kpn*I confirm the genomic maps (data not shown). The *Emv-15* alleles are aligned with respect to their mouse cellular sequences. *Emv-15*^{v} differs from *Emv-15*^{a} only by the insertion of the *Emv-15* provirus. The *Emv-15*^{v} genomic map cannot be extended as far 5′ as the *Emv-15*^{a} and *Emv-15*^{b} maps because of the presence of *Eco*RV and *Kpn*I sites in the *Emv-15* provirus. The *darkened triangle* denotes the site of integration of the *Emv-15* provirus into the *Emv-15*^{v} allele. The arrows identify restriction site differences between *Emv-15*^{a} and *Emv-15*^{b}.

Emv-15^b and *Emv-15*^{v}. A genomic restriction map of each *Emv-15* allele is shown in Figure 1B. The map is based on a comparison of genomic DNA restriction patterns from the prototype alleles (A^y, *A* and A^w, *a*) with each other and with the λ.129-A^y restriction map. *Emv-15*^{v} is the only allele that contains an ecotropic viral insertion and is associated with the A^y mutation carried by C57BL/6J-A^y/*a* and 129/Sv-A^y/A^w. *Emv-15*^{a} and *Emv-15*^{b} differ at a number of restriction endonuclease cleavage sites identified by *Hin*dIII, *Kpn*I and *Pst*I. *Emv-15*^{a} is associated with *A* and A^w carried by C3H/HeJ-*A*/*A* and 129/Sv-A^y/A^w, respectively. *Emv-15*^{b} is associated with the *a* mutation carried by C57BL/6J-*a*/*a*. The restriction map of *Emv-15*^{v} suggests that the virus integrated into a chromosome carrying *Emv-15*^{a}, since these alleles can be distinguished only by the presence of the provirus.

Correlation of *Emv-15* allelic variations with the strain origins of *agouti* locus mutations: The identification of *Emv-15* allelic variations enabled us to correlate the association of different *Emv-15* alleles with various *agouti* locus mutations carried by many different inbred strains and stocks. *Kpn*I-digested DNA from more than 50 inbred mouse strains or stocks carrying various *agouti* locus mutations was screened by Southern blot analyses with p15.4. Restriction patterns characteristic of *Emv-15*^{a}, *Emv-15*^{b} and *Emv-15*^{v} were the only detectable allelic variations. Table 2 lists the inbred strains examined, their *agouti* genotype, and the *Emv-15* genotype identified by p15.4. Table 1 summarizes the *Emv-15* allele(s) identified for each *agouti* locus mutation. The *Emv-15* alleles identified could be correlated with the known strain and allelic origin of each specific *agouti* mutation with few exceptions. Alleles A^{vy}, A^{iy}, a^m, a^x, a^{jl}, and a^e, known to have arisen from a mutation of *A* or A^w, showed the *Emv-15*^{a} restriction pattern, whereas A^{sy}, $A^{w\text{-}J}$, A^i, and $a^{t\text{-}42J}$, known to have arisen from a mutation of *a*, exhibited the *Emv-15*^{b} restriction pattern. One exception was found in SM/J mice. SM/J-A^w/*a* and SM/J-*a*/*a* mice are homozygous for *Emv-15*^{a}.

Two strains show exception to the close association of the *Emv-15* provirus with the A^y mutation: Previous investigations (COPELAND, JENKINS and LEE

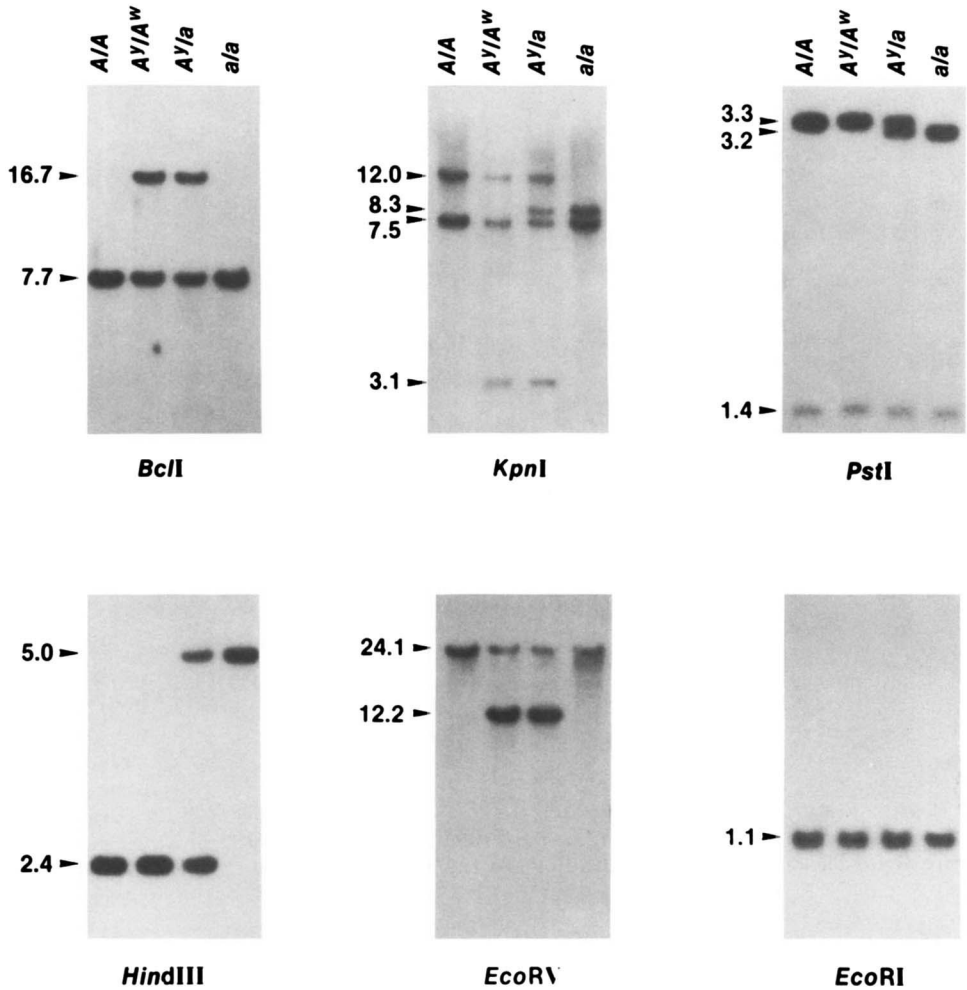

FIGURE 2. Southern blot analyses of genomic DNA from inbred strains carrying A^y, A^w, A, and a. Genomic DNAs (5 μg/lane) from C3H/HeJ-A/A, 129/Sv-A^y/A^w, C57BL/6J-A^y/a, and C57BL/6J-a/a were digested with the restriction endonucleases listed at the bottom of each panel. Southern blot analyses using p15.4 as a probe revealed the restriction fragments shown. The molecular weight (in kb) of each fragment is listed at the left of each panel.

1983) have shown that the presence of the *Emv-15* provirus is concordant with the presence of the A^y mutation in strains C57BL/6J-A^y/a, 129/Sv-A^y/A^w,and LT/Sv-A^y/a. Evidence for the close association of the *Emv-15* provirus and the A^y mutation comes from the fact that the A^y mutation was transferred onto the C57BL/6J background by 65 backcross generations and has subsequently been maintained by more than 40 generations of brother-sister matings. The C57BL/6J-A^y allele was subsequently transferred to strains 129/Sv and LT/Sv by 9 and 6 backcross generations, respectively. In addition, the 129/Sv-A^y/A^w strain has subsequently been maintained by more than 40 generations of brother-sister matings. The *Emv-15* provirus is still associated with the A^y mutation in C57BL/6J-A^y/a, 129/Sv-A^y/A^w and LT/Sv-A^y/a. The A^y mutation carried by AG/CamPa and stock 6PB most likely dates back to the A^y mutation used by C. C. LITTLE (1913), as does the A^y mutation carried by C57BL/6J (E. S. RUSSELL, personal communication). The *Emv-15* provirus is associated with the A^y mutation in strain AG/CamPA and stock 6PB, providing further evidence of the close association of the *Emv-15* provirus with the A^y mutation.

We have identified two potential exceptions to the close association of the *Emv-15* provirus with the A^y mutation. A Southern blot of genomic DNA from strains YS/Icr and YBR/Ki (both carrying the A^y mutation) was probed with the ecotropic virus-specific probe (Figure 3, top panel). The results show that the YS/Icr strain is homozygous for the *Emv-15* provirus, regardless of whether the mouse is black (a/a) or yellow (A^y/a). In contrast, the YBR/Ki mouse does not have an ecotropic MuLV insertion at the *agouti* locus, even though it carries the A^y mutation.

The *Emv-15* alleles carried by C57BL/6J-A^y/a, YS/Icr-A^y/a, YBR/Ki-A^y/a mice and their a/a sibling controls were also analyzed by Southern blot analysis using the p15.4 probe (Figure 3, bottom panel). C57BL/6J-A^y/a is heterozygous for *Emv-15*^{v} and *Emv-15*^{b}; C57BL/6J-a/a is homozygous for *Emv-15*^{b}. YS/Icr-A^y/a and YS/Icr-a/a are homozygous for *Emv-15*^{v}, whereas YBR/Ki-A^y/a and YBR/Ki-a/a are homozygous for *Emv-15*^{b}. We also examined the A^y mutation from the YBR/Wi strain; both A^y/a and a/a mice were homozygous for *Emv-15*^{b} (data not shown).

DISCUSSION

The association of an ecotropic MuLV (*Emv-15*) with the A^y mutation (COPELAND, JENKINS and LEE

TABLE 2

Summary of *Emv-15* alleles identified by p15.4 in various inbred strains and stocks carrying *agouti* alleles

Emv-15 genotype	*Agouti* locus genotype	Inbred strain or stock
Emv-15^a/Emv-15^a	*A/A*	BALB/cByJ, BALB/cJ, BALB/cWtEi, CBA/CaJ, CBA/CaH-T6J, CBA/J, CE/J, C3HeB/FeJ, C3H/HeJ, C3H/HeSnJ, C3H/Rl, HRS/J, IS/CamEi, MA/MyJ, PL/J, RIIIS/J, SEA/GnJ, SJL/J, SWR/J
	A^m/a^m	AM/Wf
	A^{vy}/a^m	AM/Wf
	A^w/A^w	LP/J, 129/J, 101/Rl
	A^w/a	SM/J
	a/a	SM/J
	A/a^{jl}	Stock 85FBFo
	a^e/a^e	AEJ/Gn, AE/Wf, AG/CamPa
Emv-15^a/Emv-15^b	*A^{vy}/a*	C57BL/6J.C3H-*A^{vy}*
	A^{iy}/a	C57BL/6J.C3H-*A^{iy}*
	a^x/a	AX/Pa
	a^{jl}/a	Stock 85FBFo
Emv-15^b/Emv-15^b	*a/a*	A/HeJ, A/J, A/WySnJ, AKR/J, Au/SsJ, AX/Pa, BDP/J, BUB/BnJ, C57BL/KsJ, C57BL/6ByJ, C57BL/6J, C57BL/10J, C57BL/10SnJ, C57BR/cdJ, C57L/J, C58/J, DBA/1J, DBA/2DeJ, DBA/2J, I/LnJ, LG/J, LT/Sv, MOR/Cv, NZB/BINJ, P/J, RF/J, SEC/1ReJ, ST/bJ, YBR/Ki
	A^y/a	YBR/Ki
	A^{sy}/a	C57BL/6J-*A^{sy}*
	A$^{w\text{-}J}$/A$^{w\text{-}J}$	C57BL/6J-*A$^{w\text{-}J}$*
	A^i/a	C57BL/6J-*A^i*
	a^t/a^t	C57BL/6J-*a$^{t\text{-}42J}$*, MWT/Le
Emv-15^v/Emv-15^a	*A^y/A^w*	129/Sv.B6-*A^y*
	A^y/a^x	Stock 6PB
	A^y/a^e	AG/CamPa
Emv-15^v/Emv-15^b	*A^y/a*	C57BL/6J.?-*A^y*, LT/Sv.B6-*A^y*
Emv-15^v/Emv-15^v	*A^y/a*	YS/Icr
	a/a	YS/Icr

1983) has provided a direct means to study DNA sequences at or near the *agouti* locus. We report the isolation of a cloned genomic DNA fragment that contains the *Emv-15* provirus along with 5′ and 3′ flanking mouse sequences. A unique sequence subclone, p15.4, was identified and used to examine the genomic region adjacent to the *Emv-15* viral insertion site. Southern blot analyses of the *Emv-15* locus in C3H/HeJ-*A/A*, 129/Sv-*A^w/A^w*, 129/Sv-*A^y/A^w*, C57BL/6J-*A^y/a* and C57BL/6J-*a/a* mice revealed three restriction endonuclease digestion patterns. Each pattern identified a unique allele of the *Emv-15* locus.

The restriction patterns of the 129/Sv-*A^w* and C3H/HeJ-*A* alleles identify *Emv-15^a*. Both agouti and white-bellied agouti phenotypes are found in feral populations [reviewed by SAGE (1981)] and therefore *A* and *A^w* are believed to represent wild-type alleles of the *agouti* locus. All inbred strains examined that carry *A* or *A^w* (excluding *A^w* alleles known to have arisen from *a*) also carry *Emv-15^a*. In addition, mice carrying *agouti* locus mutations known to have arisen from a mutation of *A* or *A^w* (*A^{vy}*, *A^{iy}*, *a^m*, *a^x*, *a^{jl}*, and *a^e*) also carry *Emv-15^a*. Therefore, the presence of *Emv-15^a* is closely associated with the presence of *A*, *A^w*, or with *agouti* mutations known to have arisen on either an *A* or *A^w* background.

Emv-15^b was defined by C57BL/6J-*a/a* mice. With few exceptions (see below), inbred strains carrying the *a* mutation also carry *Emv-15^b*. Mice carrying *agouti* locus mutations known to have originated on an *a* background (*A^{sy}*, *A$^{w\text{-}J}$*, *A^i*, and *a$^{t\text{-}42J}$*) also carry *Emv-15^b*. Therefore, *Emv-15^b* appears to be associated with *a* as well as with mutations arising from *a* in most inbred strains.

One exception to the overall correlation between specific *Emv-15* alleles and *agouti* locus alleles was found in SM/J. The SM/J strain is maintained in forced heterozygosity for *A^w* and *a*. Based on the data from Table 2, the genotype of SM/J-*A^w/a* and SM/J-*a/a* mice would be predicted to be *A^w Emv-15^a/a Emv-15^b* and *a Emv-15^b/a Emv-15^b*, respectively. However, both SM/J-*A^w/a* and SM/J-*a/a* mice are homozygous for *Emv-15^a*. The simplest explanation for these results is that a crossover occurred between the *Emv-15* locus and the *agouti* locus. A similar situation exists in YS/Icr mice. The *A^y* and *a* alleles are maintained in forced heterozygosity in the YS/Icr strain, yet this strain is homozygous for *Emv-15^v*. Again, a crossover between *Emv-15^v* and *a* could have placed these two alleles on the same chromosome and inbreeding could have resulted in fixation of the recombinant chromosome. Alternatively, SM/J and YS/Icr may carry *a* alleles that are different from the *a* alleles carried by most inbred strains.

White-bellied agouti mice can carry either *Emv-15^a* or *Emv-15^b*, depending on the origin of the mutation expressing this coat-color phenotype. The C57BL/6J-*A$^{w\text{-}J}$* allele is syntenic with *Emv-15^b*, consistent with the fact that *A$^{w\text{-}J}$* arose spontaneously on the C57BL/6J-*a Emv-15^b/a Emv-15^b* background. The *A^w* alleles of LP/J, 129/Sv, 129/J, 101/Rl and SM/J are syntenic with *Emv-15^a*. The LP/J, 129/Sv, 129/J and 101/Rl strains share a common ancestral lineage that dates back to DUNN in 1928 [reviewed by MORSE (1981)]. The origin of the *A^w* alleles in these strains is not known. Similarly, the SM/J strain was derived from a number of color stocks [reviewed by MORSE (1981)] and the origin of its *A^w* allele is not clear. The *A^w* allele of LP/J, 129/Sv, 129/J, 101/Rl and SM/J may therefore represent the fixation of a wild-type *A^w* allele. These

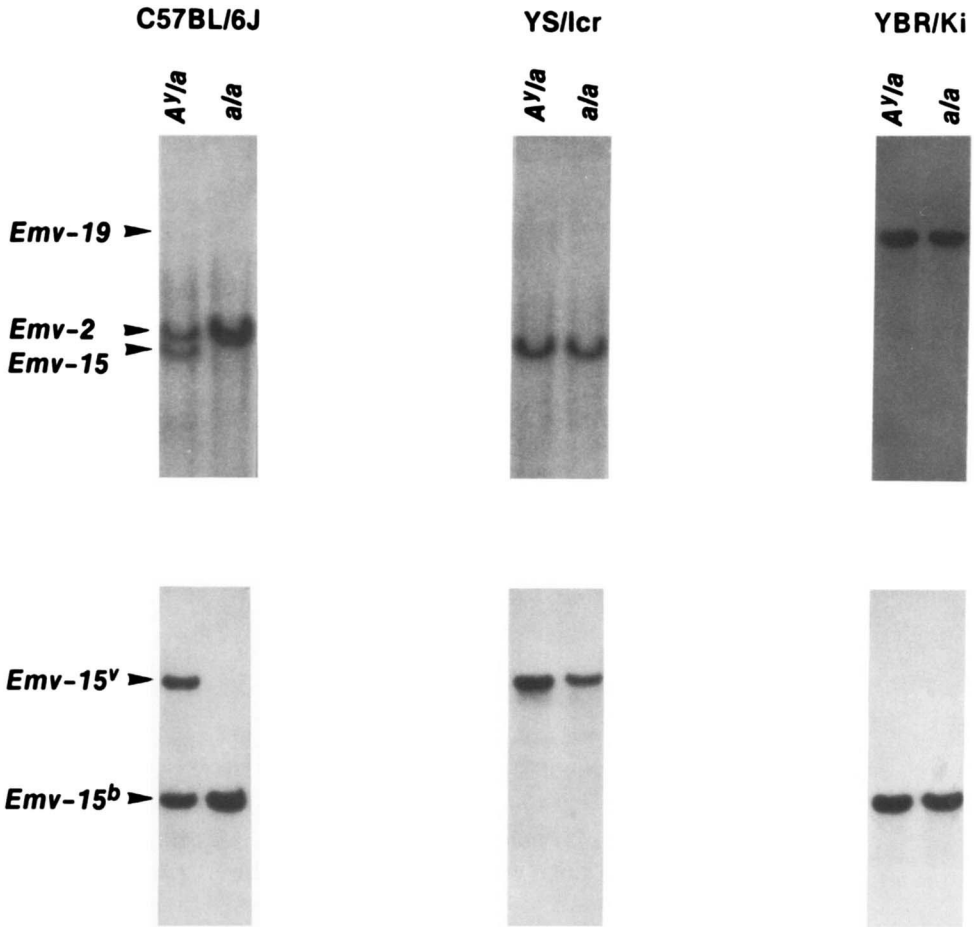

FIGURE 3. Viral content and *Emv-15* alleles carried by three inbred strains. Genomic DNAs (5 μg/lane) from C57BL/6J, Ys/Icr, and YBR/Ki mice carrying A^y/a and their *a/a* littermates, were digested and probed with *Pvu*II and the ecotropic virus-specific clone (*top panel*) or *Bcl*I and the p15.4 clone (*bottom panel*), respectively. The specific loci identified by each probe are listed to the left of both panels. The ecotropic virus-specific probe reveals two endogenous proviruses, *Emv-2* in C57BL/6J (JENKINS *et al.* 1982) and *Emv-19* in YBR/Ki, that are not specifically associated with the A^y mutation. *Hin*dIII and *Kpn*I digestions of C57BL/6J, YS/Icr, and YBR/Ki DNAs probed with p15.4 give results confirming the *Emv-15* allele assignments in the bottom panel (data not shown).

results would predict that the A^w allele can be associated with either *Emv-15*^{a} or *Emv-15*^{b} in feral populations. Preliminary analysis of laboratory colonies of wild mice has confirmed this prediction (L. D. SIRACUSA, N. A. JENKINS and N. G. COPELAND, unpublished results).

The *A* allele was always associated with *Emv-15*^{a} in the inbred strains and stocks tested. However, preliminary analysis suggests that the agouti phenotype can also be associated with *Emv-15*^{b} in wild mouse stocks (L. D. SIRACUSA, N. A. JENKINS and N. G. COPELAND, unpublished results). The *A* allele has not been found associated with *Emv-15*^{v}, possibly because of the lack of opportunity for a crossover between these two loci in inbred strains.

The a^m, a^x, a^{jl} and a^e alleles are associated with *Emv-15*^{a}. These *agouti* alleles are radiation-induced mutations of *A Emv-15*^{a} or A^w *Emv-15*^{a} chromosomes and may be the result of deletion of genomic sequences. However, Southern blot analysis of *Kpn*I-digested genomic DNA probed with p15.4 did not detect deleted or altered *Emv-15*^{a} sequences. These data also are consistent with the idea that the *Emv-15* locus is physically separated from the *agouti* locus. The fact that the *Emv-15* allele is correlated with the *agouti* allele in the strain of origin of a mutation, and not with the mutated form of the *agouti* allele, is in itself strong evidence for separate loci.

The restriction patterns of the C57BL/6J-A^y and the 129/Sv-A^y chromosomes identify *Emv-15*^{v}; the *Emv-15* provirus is present only in *Emv-15*^{v}. The restriction patterns of *Emv-15*^{a} and *Emv-15*^{v} suggest that virus integration occurred within *Emv-15*^{a}. If virus integration is responsible for the A^y mutation, then all mice tested that carry A^y should be heterozygous for *Emv-15*^{v}. However, YS/Icr-A^y/a mice are homozygous for *Emv-15*^{v}, whereas both YBR/Ki-A^y/a mice and A^y/a mice carrying the A^y mutation from the YBR/Wi strain are homozygous for *Emv-15*^{b} and do not carry the *Emv-15* provirus. The simplest explanation of the data, assuming that the A^y allele carried by YS/Icr and YBR mice is the same A^y allele carried by C57BL/6J, is that crossovers have occurred between the *agouti* alleles, A^y or *a*, and the *Emv-15* alleles, *Emv-15*^{v} or *Emv-15*^{b}, in YS/Icr and YBR mice. The resulting chromosomes carry one or the other of the two predicted reciprocal recombination products, A^y and *Emv-15*^{b}, as seen in YBR mice and, *a* and *Emv-15*^{v}, as seen in the YS/Icr strain. Further crosses [described in SIRACUSA *et al.* (1987)] are needed to confirm this result and to measure the actual map distance between the *Emv-15* locus and the *agouti* locus.

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